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Oxidative Breakdown Products of Catecholamines and Hydrogen Peroxide Induce Partial Metamorphosis in the Nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opisthobranchia)

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Abstract. Veliger larvae of the aeolid nudibranch *Phestilla sibogae* metamorphose in response to a soluble factor from their prey coral, *Porites compressa*. Metamorphosis begins with destruction of the velum, a ciliated structure used for swimming and feeding. Previous investigation had shown that *P. sibogae* larvae exposed to certain catecholamines lost the velum, but then failed to complete any subsequent steps characteristic of natural coral-induced metamorphosis. Because catecholamines oxidize rapidly in seawater, we have re-examined morphogenic effects of catecholamines using superfusion chambers that allow periodic replacement of test solutions. We report that fresh, unoxidized catecholamines do *not* induce velar loss, but that this morphogenic activity develops in aged, oxidized solutions of a variety of catecholamines and other catechol compounds. Evidence is presented that this activity is attributable to hydrogen peroxide, a byproduct of catechol autoxidation. Hydrogen peroxide induces velar loss at 10^{-4} *M*. The possible relationship of peroxide-induced velar loss to natural coral-induced metamorphosis is discussed.

Introduction

Chemical and neural mechanisms governing metamorphosis in marine invertebrates have long been of interest both to ecologists seeking to understand recruitment of larvae into adult populations, and to more reductionist biologists who view invertebrate larvae as excellent model systems for exploring the regulation of development (Hadfield, 1986). Larvae of the aeolid nudibranch *Phestilla sibogae* metamorphose upon exposure to a water-soluble factor derived from the stony coral *Porites compressa*, *P. sibogae*'s adult prey. Efforts to isolate and identify the coral-derived metamorphic inducer (CI, Hadfield and Pennington, 1990) have been accompanied by the screening of a wide range of chemical species for their capacity to induce metamorphosis (Hadfield, 1984; Hirata and Hadfield, 1986; Yool *et al.*, 1986; Pennington and Hadfield, 1989). Any such morphogens discovered by this second approach can be evaluated as possible structural analogues of CI, or as molecules involved in internal transduction of the CI signal, or as regulators of developmental mechanisms that normally unfold as a consequence of metamorphic induction by CI. Known neurotransmitters and neurohormones are among the plausible candidates for all three of these roles (D. E. Morse, this symposium; Bonar *et al.*, 1990). If a morphogenic response can be induced by application of a known neuroactive compound, certain plausible hypotheses may be made about what sorts of receptors and internal transduction systems might mediate natural metamorphosis, and appropriate experiments designed to test the implicated mechanisms.

Hadfield (1984) reported that larvae exposed to the catecholamines epinephrine (10^{-4} *M*) or norepinephrine (10^{-3} *M*) would often undergo a partial metamorphosis restricted to loss of the velum (a ciliated larval swimming and feeding organ), not followed by any of the subsequent steps in the morphogenic sequence characteristic of nat-

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Abbreviations: CI: natural coral-derived metamorphic inducer; ASW: artificial seawater; FSW: filtered seawater; EP: (−)epinephrine; NE: (−)norepinephrine; IP: (−)isoproterenol; DA: dopamine; DOPA: L-B-3, 4-dihydroxyphenylalanine; DOPAC: 3,4-dihydroxyphenylacetic acid; DOMA: 3,4-dihydroxymandelic acid; DOB: 1,2-dihydroxybenzene; HVA: homovanillic acid; OCT: octopamine.

ural metamorphosis (described in Bonar and Hadfield, 1974; Hadfield, 1978). A difficulty in the interpretation of this result was that catechols autoxidize rapidly to quinones in alkaline aqueous solutions, in a multi-step reaction that generates hydrogen peroxide (H_2O_2). Figure 1 shows the net result of this reaction; its mechanism, the nature of intermediate products and regulation by pH and various catalysts have been explored for several catecholamines and are the subject of an extensive literature (Heacock, 1959; Hawley *et al.,* 1967; Misra and Fridovich, 1972; Graham, 1978; Cohen, 1983). At 25°C, a 10^{-4} *M* solution of epinephrine in MBL artificial seawater (ASW; Cavanaugh, 1956) Tris-buffered to pH 8.2 begins to turn visibly pink within 15 min due to the appearance of the quinone oxidation intermediate, adrenochrome. Maximum adrenochrome concentration, measured spectrophotometrically, is attained within 3 h (Pires and Hadfield, unpub. data). We therefore decided to re-examine morphogenic effects of catecholamines and related compounds using superfusion chambers that permit rapid periodic replacement of test solutions to control for catechol autoxidation. Our goal was to determine whether the previously reported partial metamorphosis was indeed due to catecholamines, or to some product of catecholamine oxidation.

We report that partial metamorphosis (velar loss) is induced in *P. sibogae* by solutions of any of several catechol compounds aged in ASW or by H_2O_2 but not by fresh catecholamines. We also provide evidence that the morphogenic potency of aged catechols is due to H_2O_2 or a derivative oxygen species generated as a consequence of catechol autoxidation. These results emphasize the need for caution in interpreting biological effects of bath-applied catecholamines and other unstable chemical species. Our results also suggest testable hypotheses concerning possible roles of H_2O_2 and oxygen radicals in natural coral-induced metamorphosis.

Materials and Methods

Larval culture

All larvae used in these experiments were taken from our laboratory culture system. Adult *P. sibogae* were kept together with field-collected heads of their prey coral *P. compressa* in outdoor sea tables supplied with running unfiltered seawater (~25°C). Egg masses deposited on the coral were collected daily and transferred to .22 μm filtered seawater (FSW). Eggs developed at 25°C in aerated glass beakers in an incubator and were mechanically hatched at day 6 post-fertilization. Subsequent culture procedures were as previously described (Miller and Hadfield, 1986) except that larval culture chambers continued to be maintained in an incubator at 25°C after hatching.

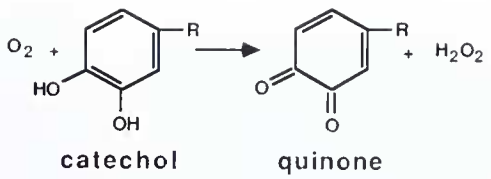

Figure 1. Generalized autoxidation of a catechol to a quinone, with the production of hydrogen peroxide (H_2O_2). For catecholamines, this reaction may also involve cyclization of the side chain R.

Experiments were conducted on 10-day-old (post-fertilization), unfed larvae. These larvae are facultative planktotrophs; under our culture conditions nearly all 10-day-old veligers are competent for metamorphosis without having to feed (Kempf and Hadfield, 1985; Miller and Hadfield, 1986).

Preparation of test solutions

(−)Epinephrine (EP), (−)norepinephrine (NE), (−)isoproterenol (IP), dopamine (DA), L-B-3, 4-dihydroxyphenylalanine (DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxymandelic acid (DOMA), catechol (1,2-dihydroxybenzene, DOB), homovanillic acid (HVA), octopamine (OCT), acetylsalicylic acid (aspirin), and thymol-free bovine catalase were purchased from Sigma Chemical Co. (St. Louis, Missouri). The first eight compounds above, all of which contain a catechol group, are sometimes referred to generically in the text as "catechol compounds" or "catechols." To avoid confusion, catechol itself is referred to as 1,2-dihydroxybenzene (DOB). All monoamines were obtained as hydrochloride salts except for EP (bitartrate) and DOPA (free acid). Pharmaceutical 3% H_2O_2 (Parke-Davis) was purchased locally. Stock solutions of test compounds were made fresh daily in deionized water at 10 times the desired final concentration and then diluted into 1.1× normal strength MBL ASW (Cavanaugh, 1956) buffered to pH 8.1–8.2 with 10 m*M* Trizma® (Sigma). This practice yielded ASW solutions of test compounds that were ionically equivalent to normal strength (1.0×) MBL ASW.

Many of the experiments presented in this paper were conducted to assay morphogenic potencies of fresh *versus* aged (oxidized) solutions of various catechol compounds. Aged solutions of test compounds in ASW were prepared as above and allowed to stand 10–14 h at 23–25°C. Fresh solutions were prepared in the same way from test compound stocks and 1.1× MBL ASW but were mixed immediately before use, as were ASW solutions of H_2O_2 for experiments on morphogenic effects of H_2O_2.

Experimental chambers

Experimental chambers (Fig. 2) were designed to permit rapid, frequent replacement of test solutions during ex-

periments. Each chamber is assembled from two symmetrical halves, constructed as follows. The body of each half-chamber is made from the base of a disposable plastic spectrophotometer cuvette cut to a volume of 1 cm^3. An 18-gauge hypodermic needle, cut to a length of 1–2 mm, is inserted through a small hole in the base of the half-chamber and cemented in place with silicone rubber aquarium sealant (Dow-Corning or equivalent). This feature allows replacement of test solutions with a syringe. Silicone rubber gaskets on the open rim of each half-chamber seal a mesh barrier (100 μm Nitex®) that separates the two halves. The halves are clamped together with rubber bands.

Our method for making the gaskets is generally useful for the construction of small watertight apparatuses. First, silicone rubber aquarium sealant is applied to the gasket-bearing surface to a slightly greater depth than the desired thickness of the finished gasket. Then a microscope slide (or other piece of smooth flat glass) smeared with a very thin film of silicone stopcock grease is laid down on the wet sealant and lightly pressed down until good contact is visible between sealant and glass along the entire surface of the gasket. After the sealant has cured, the glass can be "popped" off the finished gasket with a razor blade.

Comparisons of morphogenic potencies of fresh and aged test solutions (Figs. 4 and 6) were carried out in the superfusion chambers according to the following protocol. Each chamber was loaded with 17–112 larvae using a pipet. After the chamber halves were assembled, each chamber was flushed with 10 ml (=5× chamber volume) of test solution. Thereafter, test solutions were replaced in the same manner every 30 min until a total exposure time of 7 h had elapsed. Then the chambers were flushed with FSW and larvae washed out into Stender dishes. Larvae were scored for velar loss 16–24 h later (criteria below). In these experiments one set of 3 or 4 chambers was run with fresh test solutions (prepared as above for each solution change), while another set was run simultaneously with aged solutions (prepared as above the night before the experiment). The solution changing procedure, as well as other aspects of physical manipulation of the larvae, was identical in fresh solution and aged solution treatments.

Experiments on dose-dependent morphogenic effects of H_2O_2 and of aged DA (Figs. 5 and 7) were performed in 6 cm Stender dishes. Larvae (53–220 per dish) were exposed to several concentrations of H_2O_2 in ASW for 7 h without solution changes, and then washed into FSW for scoring as above.

Scoring of velar loss

Different degrees of velar loss are described and illustrated below. For scoring purposes, larvae able to swim freely and climb through the water column were considered to be intact. Such individuals never showed any evidence of velar reduction when examined at 50× magnification. Larvae lying on the bottom of the dish or swimming, but failing to clear the bottom, were scored as "velum lost" if any of the large ciliated cells at the velar margin were missing, or if the velar lobes were noticeably shortened. Instances of partial (Fig. 3B) and complete (Fig. 3C) velar loss were combined and divided by the total number of larvae to obtain a frequency of velar loss for each trial.

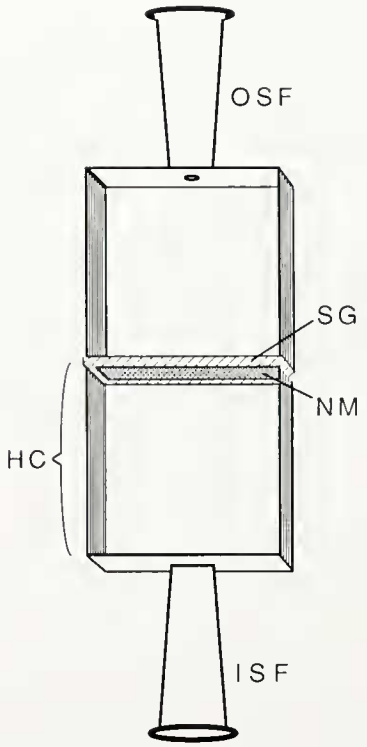

Figure 2. Superfusion chamber used in experiments comparing morphogenic potencies of fresh *versus* aged solutions of catechols. Larvae are retained in the lower half-chamber (HC) by a Nitex mesh barrier (NM), held in place by silicone rubber gaskets (SG). Inlet and outlet syringe fittings (ISF and OSF) allow periodic flushing with a syringe containing test solution. Total chamber volume is 2 cm^3. Half-chambers are held together during use by rubber bands (not shown).

Results

Induction of velar loss by aged catechols and H_2O_2

Loss of the velum, one of the major morphological transformations occurring during metamorphosis, can be considered a partial metamorphosis (Bonar and Hadfield, 1974; Hadfield, 1984). We consistently found that high proportions of larvae lost some or all of the velum (except for small remnant clumps of cephalic supportive cells) when exposed for 7 h to aged solutions of any of the cat-

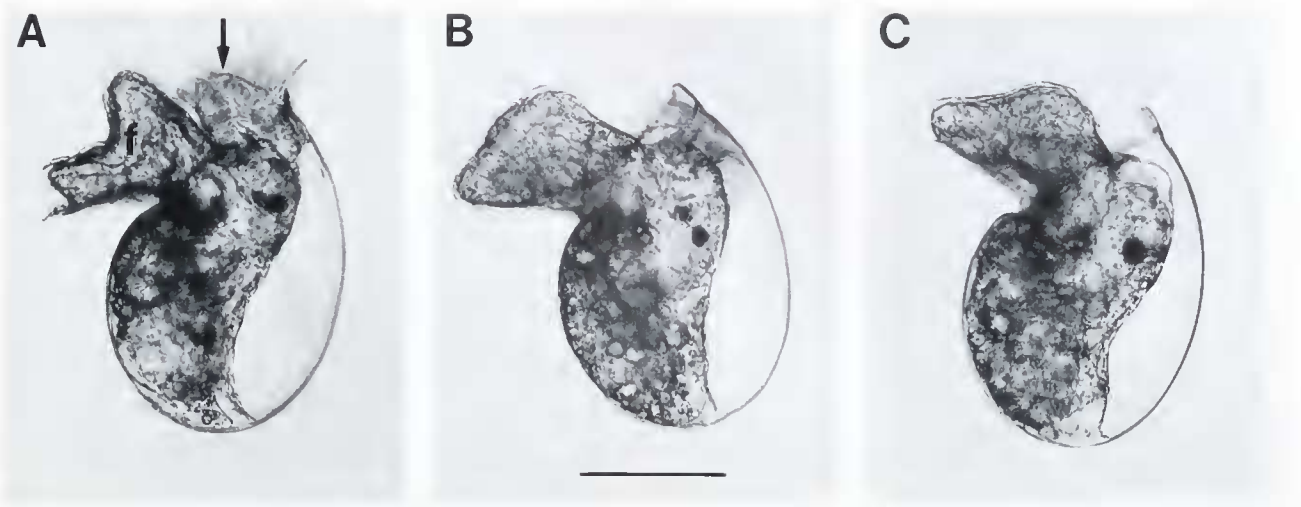

Figure 3. Velar loss in *Phestilla sibogae*. A. Lateral view of 11-day-old, untreated larva. Animal was photographed with intact velum (arrow) partly withdrawn into the larval shell to keep velum in same plane of focus as the rest of the animal; foot (f) is partly extended. Outlines of large ciliated cells are visible along the velar margin. B. Partial velar loss in an 11-day-old larva treated with 10^{-4} *M* H_2O_2 on day 10. C. Complete velar loss in an 11-day-old larva treated with 2×10^{-4} M H_2O_2 on day 10. Differences between larvae in shape of foot represent a range of movement independent of velar loss. Scale bar = 100 μm.

echol compounds tested, or to H_2O_2, but not when exposed to fresh catecholamines. Figure 3 illustrates velar loss in response to H_2O_2 but it could just as well indicate the results obtained with aged solutions of catechols. An 11 day-old untreated larva with intact velum is shown in Figure 3A. Velar loss induced by H_2O_2 or by aged catechols (or by Cl) begins with the detachment of the large ciliated cells at the velar margin. These cells are cast off intact, and their cilia continue to beat for some time after detachment. After separation of these cells begins, regression of non-ciliated supportive cells of the velar lobes becomes apparent. This state of partial velar loss is depicted in Figure 3B. This state is stable in that velar loss will proceed no further in larvae washed out of the H_2O_2 or aged catechol treatment into FSW. If the above treatments are applied in sufficiently high concentration (see below), 7-h treatment results in detachment of all the large ciliated velar cells and detachment or regression of remaining tissues, leaving small cephalic mounds of supportive cells where the velar lobes had been (Fig. 3C). Velar loss in response to these treatments, like that seen in natural coral-induced metamorphosis, is highly tissue-specific. Other ciliated epithelia (of the foot, for example) remain intact. When velar loss is induced with H_2O_2 or aged catechols, metamorphosis does not proceed beyond this point. However, if such larvae are then exposed to Cl, many will right themselves on the foot, take up the settled posture characteristic of natural metamorphosis (Hadfield, 1978), and complete metamorphosis in an apparently normal fashion.

Comparison of effects of fresh and aged catechols

Morphogenic effects of fresh and aged solutions of the catecholamines EP, NE, and IP (2×10^{-4} *M*) and DA (10^{-4} *M*) were quantitatively compared (Fig. 4). Dramatically different results were obtained in parallel trials using aged and fresh solutions of the same compounds, replaced every 30 min during the 7-h exposure period. Most larvae lost some or all of the velum after exposure to aged catecholamines. After exposure to fresh catecholamines, larvae rarely showed any indication of velar

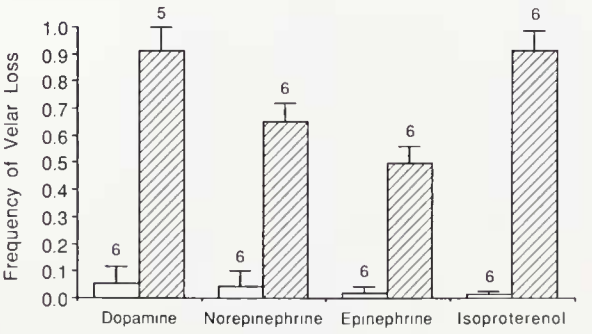

Figure 4. Frequencies of velar loss after 7-h exposure to fresh (open bars) or aged (hatched bars) solutions of catecholamines. Concentrations are 2×10^{-4} *M* except for dopamine (10^{-4} *M*). Ordinate values and error bars are means and standard deviations, respectively, calculated from arcsine transformed data. Numbers above error bars indicate the number of replicate trials, each involving a chamber containing 17–96 larvae. Trials for each compound were conducted on at least two different batches of larvae.

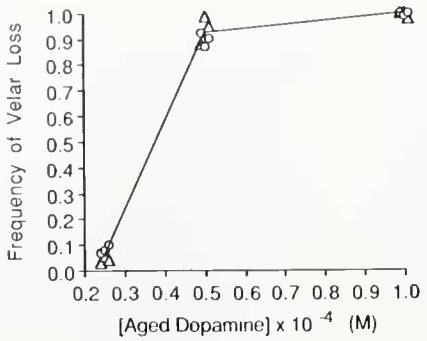

Figure 5. Frequencies of velar loss after 7-h exposure to varying concentrations of aged dopamine solutions. Triangles and circles represent two assays conducted in triplicate on two different batches of larvae. Each symbol represents a trial involving 53–220 larvae. Lines connect grand means of velar loss at each concentration, calculated from arcsine transformed data.

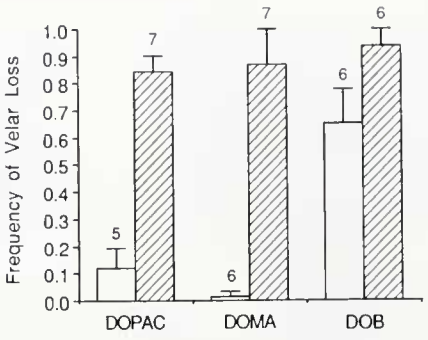

Figure 6. Frequencies of velar loss after 7-h exposure to fresh (open bars) or aged (hatched bars) solutions of the catechol compounds dihydroxyphenylacetic acid (DOPAC), dihydroxymandelic acid (DOMA), and dihydroxybenzene (DOB). Concentrations are 2×10^{-4} *M* except for DOB (10^{-4} *M*). Ordinate values and error bars are means and standard deviations, respectively, calculated from arcsine transformed data. Numbers above error bars indicate the number of replicate trials, each involving a chamber containing 18–112 larvae. Trials for each compound were conducted on at least two different batches of larvae.

loss and were generally indistinguishable in morphology and behavior from untreated animals. Larvae exposed to a higher concentration of fresh DA (2×10^{-4} *M*) according to this protocol did sometimes metamorphose completely by the time the experiment was scored, but at low frequency (0–.25, typically .10–.15), as suggested by earlier experiments conducted without solution replacement (Hadfield, 1984). However, in the current work, we used 10^{-4} *M* DA for the aged *versus* fresh comparison because aged solutions at higher concentrations proved somewhat toxic under these conditions. Complete metamorphosis was observed at very low frequency (≤.05) after exposure to fresh 10^{-4} *M* DA.

The frequency of velar loss in response to varying concentrations of aged DA is given in Figure 5. Concentration threshold for velar loss after 7-h exposure to fresh DA appears to lie between .25 and .5 $\times 10^{-4}$ *M*.

Experiments to test morphogenic effects of other catechol compounds yielded similar results (Fig. 6). Aged solutions of the deaminated catecholamine metabolites DOPAC or DOMA (both 2×10^{-4} *M*) as well as of DOB (10^{-4} *M*) consistently yielded high frequencies of velar loss. Fresh solutions of DOPAC and DOMA were relatively ineffective. Fresh DOB caused most larvae to show some evidence of velar loss, but this was invariably confined to the loss of a few large ciliated cells at the velar margin. Aged DOB, in contrast, nearly always resulted in detachment of all the ciliated velar cells and substantial regression of the velar lobes. DOPA, a catechol amino acid precursor of the catecholamine neurotransmitters, also caused a high mean frequency of velar loss (.67) in aged 10^{-4} *M* solutions, but a quantitative comparison could not be made with fresh solutions. Larvae treated with fresh DOPA tended to withdraw completely into the shell, and although vela appeared to be intact, accurate scoring of velar condition was not possible. OCT (NE minus one ring hydroxyl group) and HVA (DOPAC with one ring hydroxyl group methylated) do not oxidize as easily as their related catechol compounds, and had no morphogenic effects in aged or fresh 2×10^{-4} *M* solutions.

Quantification of H_2O_2-induced velar loss and abolition of effects of H_2O_2 and aged catechols by catalase

Because autoxidation of catechols in water yields H_2O_2 (Graham *et al.*, 1978), we tested the ability of H_2O_2 in ASW solutions to induce velar loss. Exposure to H_2O_2 for 7 h reliably induced velar loss at a concentration threshold in the range of .25–.5 $\times 10^{-4}$ *M* (Fig. 7). Solutions of .25 $\times 10^{-4}$ *M* H_2O_2 were never sufficient to cause observable velar loss; these animals were indistinguishable from untreated individuals (Fig. 3A). A large but variable fraction

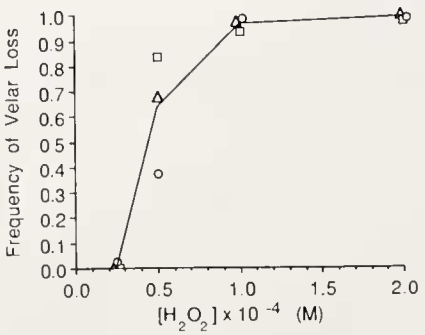

Figure 7. Frequencies of velar loss after 7-h exposure to varying concentrations of H_2O_2. Triangles, circles and squares represent three assays conducted on three different batches of larvae. Each symbol represents a trial involving 54–123 larvae. Lines connect grand means of velar loss at each concentration, calculated from arcsine transformed data.

of larvae tested at $.5 \times 10^{-4}$ *M* H_2O_2 showed clear indications of partial velar loss. In 10^{-4} *M* H_2O_2 nearly all larvae experienced at least partial velar loss; a typical instance is shown in Figure 3B. In 2×10^{-4} *M* H_2O_2, most larvae lost the entire velum except for small mounds of cephalic supportive cells (Fig. 3C).

Morphogenic potencies of H_2O_2 and aged solutions of all of the above catechol compounds were completely abolished by 10 min incubation with purified bovine catalase (5 μg/ml), prior to addition of larvae. The presence of H_2O_2 in aged solutions of catechols was confirmed by measuring an increase in dissolved oxygen concentration upon catalase treatment, with a Clark-type oxygen meter. (Catalase catalyzes the decomposition of H_2O_2 to water and molecular oxygen.) Hydrogen peroxide-induced velar loss was not inhibited by acetylsalicylic acid (aspirin) in any concentration between 10^{-6} and 10^{-3} *M*. [Aspirin, an inhibitor of prostaglandin endoperoxide synthetase, inhibits H_2O_2-induced spawning in the abalone *Haliotis rufescens* (Morse *et al.*, 1977).]

Discussion

Relationship of velar loss induced by H_2O_2 and by aged catechols to velar loss in natural metamorphosis

Velar loss in larvae of *P. sibogae* can be induced by application of H_2O_2 or aged solutions of catechols in the tenth-millimolar concentration range (Figs. 3–7). The stoichiometry of catechol autoxidation (Fig. 1), together with our observation that similar concentrations of H_2O_2 or aged catechols are required to induce velar loss, suggests the hypothesis that morphogenic activity of aged catechols is due to H_2O_2 produced upon autoxidation or to some other reactive species derived from H_2O_2 such as the hydroxyl radical $HO^{\bullet}$ (for discussions of H_2O_2 metabolism and oxygen radical biochemistry see Fridovich, 1978; Imlay and Linn, 1988; Cadenas, 1989; Kontos, 1989; Gutteridge *et al.*, 1990). Direct evidence for this hypothesis is the fact that morphogenic activity of aged catechol solutions is lost on incubation with catalase, an enzyme that selectively degrades H_2O_2 to molecular oxygen and water. However, we have not yet rigorously excluded cooperative effects of quinone oxidation products of catechols in velar destruction.

Natural coral-induced metamorphosis is preceded by settlement behavior in which the larva takes up a characteristic posture, attached by the foot to the substratum (Bonar and Hadfield, 1974; Hadfield, 1978). This behavior is not elicited by H_2O_2 or aged catechols. In natural metamorphosis, velar loss ensues in this settled position. Larvae treated with H_2O_2 or aged catechols begin to lose the velum while swimming; after enough large ciliated velar cells have been lost, larvae sink to the bottom of the experimental chamber and typically lie on a side of the shell in an extended posture.

Although the behavioral contexts for natural and artificially induced velar loss are different, the morphological phenomena share several common features. Both begin with the detachment of the large ciliated cells at the velar margin. Natural and artificially induced velar loss are both highly tissue-specific in that cell separation and tissue regression are confined to the velum and are not manifested in other ciliated epithelia. Following loss of the ciliated velar cells, clumps of nonciliated supportive cells remain as cephalic mounds (Fig. 3C). Partial metamorphosis induced by H_2O_2 or aged catechols does not proceed beyond this point. However, no loss of metamorphic competence has occurred, because such larvae can resume metamorphosis once exposed to Cl.

Further experiments are required to test the hypothesis that H_2O_2 or derivative oxygen radicals mediate velar loss in natural metamorphosis. Several techniques exist that are potentially applicable to detection of H_2O_2 or oxygen radical production in metamorphosing tissues (Freeman and Crapo, 1981; Radzik *et al.*, 1983; Ruch *et al.*, 1983; Kontos, 1989). Chemical scavengers of free radicals and of H_2O_2 can also be used to interfere with radical-dependent mechanisms (Cadenas, 1989; Kontos, 1989). We were unable to inhibit coral-induced metamorphosis with catalase, but that result is difficult to interpret because catalase is not expected to penetrate cells to reach potential sites of endogenous H_2O_2 generation or action. Future experiments will include other more penetrant scavengers. It is important to note that even if H_2O_2 or oxygen radicals are implicated in the mechanism of natural metamorphosis, there are many biological sources of these oxygen species other than oxidation of catechols (Cohen, 1983; Kontos, 1989).

Possible modes of action of H_2O_2

Hydrogen peroxide is well-known for its cytotoxic properties, which render it useful as a topical disinfectant. Mechanisms of cell damage by H_2O_2 and oxygen radicals have been subjects of several recent discussions (Imlay and Linn, 1988; Kontos, 1989; Gutteridge and Halliwell, 1990). Indeed, significant lysis of certain cultured vertebrate epithelial cells occurs upon exposure to H_2O_2 concentrations only slightly higher than those demonstrated to cause velar loss in the present study (Hayden *et al.*, 1990; Polansky *et al.*, 1990). However, the question of whether H_2O_2-induced velar disintegration in *Phestilla* larvae is a cytotoxic response remains. The facts that ciliated cells appear to be shed intact with cilia still beating and that other epithelial tissues show no evidence of injury at morphogenically active concentrations, might argue otherwise. Close structural examination of shed velar cells

in both H_2O_2-induced and coral-induced velar loss will be instructive in this regard. Non-toxic regulatory effects of H_2O_2 mimic the effects of insulin on glucose, carbohydrate, and lipid metabolism in vertebrate cells, possibly by stimulating phosphorylation of the insulin receptor (Heffetz *et al.*, 1990). This mechanism of action may warrant investigation in *Phestilla*, particularly in light of the recent discovery of a preproinsulin-related peptide in growth-regulating neuroendocrine cells of the gastropod *Lymnaea stagnalis* (Smit *et al.*, 1988) and the growing appreciation of insulin's role in cellular differentiation during embryogenesis (Alemany *et al.*, 1990). In another gastropod, the abalone *H. rufescens*, H_2O_2 induces spawning, probably by activating prostaglandin endoperoxide synthetase (Morse *et al.*, 1977). This particular pathway is unlikely to be involved in velar loss in *Phestilla* because H_2O_2 induction of velar loss is not blocked by aspirin, a potent inhibitor of that enzyme. One might speculate that H_2O_2 could also regulate the activity of a factor involved in epithelial cell adhesive interactions, perhaps akin to the "scatter factor" recently described as a promoter of cell-cell separation in cultured mammalian epithelia (Stoker and Gherardi, 1989).

Implications for other taxa

Our study points out the need for caution in the interpretation of behavioral and morphogenic effects of bath-applied catecholamines on marine animals. In the bivalve *Crassostrea gigas*, sufficient physical controls and corroborating pharmacological evidence have been marshalled in support of the hypothesis that dopaminergic and adrenergic neural pathways, mediate settlement and morphogenesis, respectively (Coon and Bonar, 1987; Bonar *et al.*, 1990). There are also brief reports of metamorphic induction by DOPA in the mussel *Mytilus edulis* (Cooper, 1982), and by DA in the mud snail *Ilyanassa obsoleta* (Levantine and Bonar, 1986), but details of the methods are not given. Certain catecholamines have been reported to induce metamorphosis in the scallops *Patinopecten yessoensis* (Kingzett *et al.*, 1990) and *Pecten maximus* (Cochard *et al.*, 1989), but in both of these studies the problem of catechol oxidation was not thoroughly resolved. DOPA induces a low frequency of metamorphosis in the polychaete *Phragmatopoma californica* (Jensen, 1987), but that work has implicated cross-linked quinoid derivatives of DOPA residues in proteins, rather than catecholamine neurotransmitters, in the inductive pathway (Jensen and Morse, 1990). To our knowledge the only other published example of catecholamine induction of larval metamorphosis is in the echinoid *Dendraster excentricus* (Burke, 1983). Whole larvae and excised larval arms metamorphosed in response to DA, but not to EP or NE. The response specificity suggests that DA did not act as a source of H_2O_2 in those experiments, but the protocols are not detailed enough to permit clear resolution of this issue.

Our results should not be interpreted to mean that catecholamines do not act as neurotransmitters mediating metamorphosis in *P. sibogae*. Bath-applied substances might not reach their target tissues in the proper concentration, or the co-activation of receptors on many cells throughout the nervous system may result in net inhibition of circuits that effect metamorphosis. We do hope that this study sounds a cautionary note to others investigating chemical control of metamorphosis, and prompts consideration of a possible morphogenic role for H_2O_2.

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